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(54) **A test set and a process for the determination of antibiotics in milk and a novel streptococcus thermophilus strain to be used therein.**

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JOURNAL OF DAIRY RESEARCH, vol. 50, 1983, pages 185-191, London, GB; S.A. THOROGOOD et al.: "An evaluation of the Charm Test - a rapid method for the detection of penicillin in milk"

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Description

The invention relates to a test set suitable for the determination of antibiotics in milk. The invention is also concerned with a novel Streptococcus thermophilus strain to be used in the test set and a process for the determination of antibiotics in milk by means of said test set.

In many situations it is of vital importance to be able to detect the presence of small amounts of antibiotics. This is the case in food industries, for instance, where the increased use of antibiotics and chemotherapeutic substances in the treatment of animals has created a need for a simple, reliable and sensitive process of determination. Since antibiotics are used also in the treatment of dairy cows and since antibiotic residues in milk may both cause health hazards and be disadvantageous for food technological reasons, it is especially important to develop processes suitable for an accurate and rapid screening of milk.

Antibiotic residues in milk are generally detected by microbiological processes which utilize the fact that bacteria are able to produce acid, reduce colours and produce growth on an agar medium. These processes are based on the bactericidal, inhibitory and morphological effect of antibiotics on certain microorganisms.

The Thermocult disk technique is an agar diffusion technique which is widely used in Finland and accepted as an official antibiotic determination procedure. In this technique the test organism is B. stearothermophilus var. calidolactis. It has been developed on the basis of an IDF standard process (IDF 1970. Detection of Penicillin in Milk by a Disk Assay Technique. International Standard FIL-IDF 57. Brussels).

A process of corresponding sensitivity is disclosed by van OS et al., Diffusion Test for the Determination of Antibiotic Residues in Milk. Neth. Milk and Dairy J. 29 (1975) 16. The Delvotest process, too, uses B. stearothermophilus var. calidolactis as the test organism. A sample (0.1 ml) is pipetted on agar contained in an ampoule, and a tablet containing nutrients and a pH indicator is added to the ampoule. The process is based on the acid producing capability of the test organism. The ampoules are incubated at 64 °C for 2.5 hours. The evaluation is based on the colour change of the agar layer.

Standard techniques further include the Intertest (BCP-Test). The test microbe used in the process is Str. thermophilus. A test tablet containing a lyophilized culture of the test microbe, nutrients, and a pH indicator (bromocresol purple) is added to a milk sample. The incubation time is 4 hours at 45 °C. If the sample does not contain any antibiotic, the colour of the solution turns from blue to green and further to yellow. The amount of the antibiotic can be determined to some extent on the basis of the colour by comparing to a colour map (THOROGOOD et al., An Evaluation on the Charm Test - A Rapid Method for the Detection of Penicillin in milk. J. Dairy Research 50 (1983) 185).

A drawback of these processes is their insufficient sensitivity in view of the needs of milk technology.

The determination of antibiotic residues in milk by means of chemical or physico-chemical processes is considerably less usual than the use of microbiological processes. Colorimetric and chromatographic processes require skilled labour and often a complicated and expensive analyzing equipment. The processes are seldom suitable for routine analyses.

The Charm test (CHARM, S.E., A 15-minute Assay for Penicillin and other Antibiotics. Cultured Dairy Products J. 14 (1979) 24) is based on the detection of radioactivity. A lyophilized culture of B. stearothermophilus culture and lyophilized ^{14}C-labelled penicillin are added to a sample. The amount of ^{14}C contained in the bacterium cells is detected by a Geiger counter; the lower the penicillin concentration of the sample, the higher is the reading of the Geiger counter. The detection time is only 15 minutes and the sensitivity of the process is 0.005 I.U. of penicillin per ml. This process, either, is not suitable for routine use; it is expensive and complicated and requires skilled persons and expensive equipment to be carried out.

Thus there is still a practical need for a sensitive process which is as broad-spectrum as possible. The process should also be simple and it should be possible to carry out the process by an equipment ready for use, whereby the test does not require skilled persons, but can be carried out e.g. on a farm.

These advantages are obtained by means of a test set according to the invention, which is characterized in that it comprises a Streptococcus thermophilus T101 concentrate and a water-based protective agent in a dilution ratio of 4 to 5 x 10^{-2}. The determination is carried out according to the invention by adding a sample to a test set comprising a Streptococcus thermophilus T101 concentrate and a water-based protective agent in a dilution ratio of 4 to 5 x 10^{-2}, and possibly an indicator, and if the test set does not comprise an indicator, an indicator is added, too; incubating the test set and the sample at 38 to 42 °C for about 4 hours; and evaluating the colour.

The invention is based on the novel Streptococcus thermophilus T101 strain, which has been isolated at the Lammi creamery of Valio. The strain has been deposited at the Deutsche Sammlung von Mikroorganismen under the deposit number DSM 4022 on March 3, 1987, and it possesses the following properties:

- gram positive,
- forms long coccus chains
- growing temperature: growth at 50 °C no growth at 10 °C
- salt resistance growth at a NaCl concentration of 2% no growth at a NaCl concentration of 6.5%,
- titrated acidity 25 to 29 °SH after 7-hour incubation at 42 °C (sterilized 10% milk powder milk)
- lactic acid %: 0.8% (incubated 2 days at 42 °C, from milk powder milk)
- fermentates lactose, saccharose and glucose.

Judging from the values given in the prior art, the novel microorganism strain is clearly more sensitive than known Streptococcus thermophilus strains, especially to penicillin and oxytetracycline.

The test set is prepared in the following way: The microorganism strain is grown in a fermentor at a pH of 6.2 to 6.5 and at 38 to 42 °C in a culture medium based on whey permeate. The growth is observed and the growth is arrested at the end of the logarithmic growth phase, whereafter the culture broth is concentrated by filtrating to a 20-fold concentration. The concentrate is measured in a dilution ratio of about 4 to 5 $\times$ 10^{-2}, preferably about 5 $\times$ 10^{-2}, into a protective agent. The protective agent may consist of water-based protective agents used in the preparation of lyophilized microbe preparations. Preferably the protective agent is an aqueous solution comprising 1.1% of sodium glutamate, 1.1% of ascorbic acid, and optionally 7% of lactose, and the pH of which is 6.5. The indicator can be added to the protective agent, or it can be added to the test set in connection with the determination. The indicator is e.g. an acid-base indicator, such as bromocresol purple. The concentrate is measured into a vessel which may be a conventional ampoule, a sealable test tube, a sample bottle, or the like. The vessel is cooled in a carbonic acid-sulphite alcohol bath, whereafter it is lyophilized and stored under vacuum. The finished test set contains about 1 to 2 $\times$ 10^6 bacteria per ml.

The antibiotic determination is carried out by adding a liquid sample and possibly an indicator to the test set. The test set and the sample are incubated and the colour changes are observed. If the sample contains antibiotics, the microorganisms in the test set are not able to grow and the colour does not change. On the other hand, if the sample does not contain antibiotics, the microorganisms grow and induce a colour change while growing.

The sensitivity of the process according to the invention was compared with the corresponding commercial THERMOCULT (Orion Diagnostica) and DELVOTEST P (Gist-Brocades) techniques and the CHARM II technique. The sample consisted of milk preheated at 95 °C for 5 minutes and the determinations were carried out according to the instructions given by the manufacturers. The results are presented in the following table, from which further appears the data given by the manufacturer Intervet concerning the

INTERTEST. The results show that the process according to the invention is more sensitive than the other processes and detects clearly the presence of all types of antibiotics/combinations.

TABLE

Experimentally determined antibiotic sensitivities (µg/ml) of the tested determination processes

ANTIBIOTIC	PROCESS ACCORDING TO THE INVENTION		THERMOCULT		DELVOTEST P		INTERTEST b)	CHARM TEST II
	A	B	own determination	(S&S) a)	own determination	(S&S) a)		
PENICILLIN	0.001-0.002 I.U.	0.001-0.002 I.U.	0.006-0.0075	0.005-0.0075	0.0025	0.0025	0.005	0.003
STREPTOMYCIN	1.25	0.25-0.4	5.0	2.5-5.0	5.0	2.5-5.0	5.0	0.1
TETRACYCLINE	0.05-0.1	0.05	0.2 c)	0.1	0.2	0.1	0.5	0.2
OXYTETRACYCLINE	0.1	0.05	0.2 c)	0.1	0.2	0.1	0.2	
AMPICILLIN	0.01	0.003	0.01 c)	0.005	0.01 c)	0.005	0.005	
ERYTHROMYCIN	0.01-0.05	0.01	0.1 c)	0.5-0.75	0.1 c)	0.75-1.0	0.1	0.01
CHLORAMPHENICOL	0.1-0.5	0.1	1.0 c)	7.5	1.0 c)	7.5-10.0	1.0	0.05
NEOMYCIN	0.5	0.1-0.2	0.5	1.0	1.0	0.5	20.0	
STREPTOMAX (penicillin +streptom.)	0.004 I.U. PEN +0.001 µg/ml STREPTOM.		0.01 I.U. PEN +0.008 µg/ml STREPTOM.					
MASTALONE (oxytetracycline+the others)	0.1	0.05	0.2 c)					

A indicator added in connection with the test (duration of the test 4 hours)
B indicator added before freeze drying (duration of the test 5 hours)
a) Sandström & Sivelä, 1984, Karjantuote 4
b) concentrations given by the manufacturers
c) could not be determined

Example 1

Preparation of the test set

Bacteria of the Streptococcus thermophilus T101 strain are inoculated in a culture medium having the following composition:
5% of whey permeate powder
1.5% of casein hydrolysate
0.5% of tryptone
1% of yeast extract
The culture medium has been sterilized at 120°C for 15 to 20 minutes, and its pH is 6.4 after the sterilization.

The test strain is grown in a fermentor at a pH of about 6.2 and at about 42°C, and the growth is monitored by observing the turbidity of the culture broth. At the end of the logarithmic growth phase the growth is arrested and the culture broth is concentrated by filtrating using a Millipore Pellicon filtration unit (0.45 µm) to a 20-fold concentration, whereby the bacterium concentration of the concentrate is about 2×10^9 bacteria per ml. The concentrate is washed with a small amount of protective agent, and about 5 ml is added to 100 ml of protective agent, whereto is possibly also added 1 ml of bromocresol purple colour (a 0.8% solution). The bacterium concentration of the solution so obtained is about 1×10^8 bacteria per ml. 1 ml of the solution is added to a conventional 10 ml ampoule which withstands drying and can be closed by vacuum. The ampoule is cooled rapidly (20 to 60 s) in a -60°C carbonic acid-sulphite alcohol bath, whereafter it is freeze dried and vacuum closed for storage. The ampoule thus prepared contains 1 to 2×10^6 bacteria per ml.

Example 2

Determination of antibiotics in a milk sample

Raw milk is heated at 95°C for 5 minutes. 2 ml of milk and possibly 20 µl of a colour indicator are added to a test set prepared according to Example 1. The test set is incubated for 4 hours at about 42°C and the colour is evaluated. Milk prepared from sour milk powder and heated at 95°C for 5 minutes is used as a control. If the milk contains antibiotics, the colour turns blue. Yellow indicates a negative result.

Claims

1. A test set suitable for the determination of antibiotics in milk, **characterized** in that it comprises a Streptococcus thermophilus T101 (DSM 4022) concentrate and a water-based protective agent in a dilution ratio of 4 to 5×10^{-2}.

2. A test set according to claim 1, **characterized** in that the protective agent comprises an aqueous solution comprising 1.1 % of sodium glutamate, 1.1% of ascorbic acid, and optionally 7 % of lactose, and the pH of which is 6.5.

3. A test set according to claim 1, **characterized** in that it further comprises an indicator.

4. A test set according to claim 3, **characterized** in that the indicator is bromocresol purple.

5. Streptococcus thermophilus T101, DSM 4022.

6. A process of determinating antibiotics in milk, **characterized** by the steps of adding a sample to a test set comprising a Streptococcus thermophilus T101 (DSM 4022) concentrate and a water-based protective agent in a dilution ratio of 4 to 5×10^{-2} and possibly an indicator, and if the test set does not contain an indicator, an indicator is also added; incubating the test set and the sample at 38 to 42°C for about 4 hours; and evaluating the colour.

Revendications

1. Composition qui convient pour la détermination des antibiotiques dans du lait, caractérisée en ce qu'elle comprend un concentré de Streptococcus thermophilus T101 (DSM 4022) et un agent protecteur à base d'eau en un rapport de dilution de 4 à 5 x 10^{-2}.

2. Composition suivant la revendication 1, caractérisée en ce que l'agent protecteur comprend une solution aqueuse contenant 1,1% de glutamate de sodium, 1,1% d'acide ascorbique et, le cas échéant, 7% de lactose et son pH est de 6,5.

3. Composition suivant la revendication 1, caractérisée en ce qu'elle comprend en outre un indicateur.

4. Composition suivant la revendication 3, caractérisée en ce que l'indicateur est du pourpre de bromocrésol.

5. Streptococcus thermophilus T101, DSM 4022.

6. Procédé de détermination des antibiotiques dans du lait, caractérisé par les stades d'addition d'un échantillon à une composition comprenant un concentré de Streptococcus thermophilus T101 (DSM 4022) et un agent protecteur à base d'eau en un rapport de dilution de 4 à 5 x 10^{-2} et, le cas échéant, un indicateur, et si la composition ne contient pas un indicateur, un indicateur est également ajouté; d'incubation de la composition et de l'échantillon entre 38 et 42°C pendant 4 heures environ; et d'évaluation de la coloration.

Patentansprüche

1. Testgarnitur, geeignet zur Bestimmung von Antibiotica in Milch, dadurch gekennzeichnet, dass sie ein Streptococcus thermophilus T101 (DSM 4022)-Konzentrat und ein Schutzmittel auf Wassergrundlage in einem Verdünnungsverhältnis von 4 bis 5 x 10^{-2} enthält.

2. Testgarnitur nach Anspruch 1, dadurch gekennzeichnet, dass das Schutzmittel eine wässerige Lösung enthält, welche 1,1 % Natriumglutamat, 1,1 % Ascorbinsäure und gegebenenfalls 7 % Lactose enthält und deren pH 6,5 beträgt.

3. Testgarnitur nach Anspruch 1, dadurch gekennzeichnet, dass sie ferner einen Indikator enthält.

4. Testgarnitur nach Anspruch 3, dadurch gekennzeichnet, dass der Indikator Bromcresolpurpur ist.

5. Streptococcus thermophilus T101, DSM 4022.

6. Verfahren zur Bestimmung von Antibiotica in Milch, gekennzeichnet durch die Stufen des Zusatzes einer Probe zu einer Testgarnitur, welche Streptococcus thermophilus T101 (DSM 4022)-Konzentrat und ein Schutzmittel auf Wassergrundlage in einem Verdünnungsverhältnis von 4 bis 5 x 10^{-2} und gegebenenfalls einen Indikator enthält, und wenn die Testgarnitur keinen Indikator enthält, wird auch ein Indikator zugesetzt; des Inkubierens der Testgarnitur und der Probe bei 38 bis 42°C während etwa 4 Stunden und der Bewertung der Farbe.

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(54) Process for the preparation of a unit for the determination of residues of antibiotics and sulphas in biological liquids and such units.

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US - A - 3 126 325

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CHEMICAL ABSTRACTS, Vol. 79, no. 17, 29 October 1973, abstract 103722x, page 311, COLUMBUS, OHIO, USA, R. GUDDING et al.: "Detection of sulfonamides in milk"

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Acta Vet. Scand. 17 (1976), pages 458-464 (Gudding)

The file contains technical information submitted after the application was filed and not included in this specification

Process for the preparation of units for the determination of residues of antibiotics and sulphas in biological liquids, and such units

The invention relates to a process for the preparation of units for the determination of residues of antibiotics and sulphas in biological liquids, such as milk, meat juice, serum and urine. The invention also relates to the units.

A similar process has been described by Gudding, Acta Vet. Scand. 17 (1976) pages 458 to 464, using plates with an agar medium in the manner as described by Galesloot et al, Netherlands Milk and Dairy Journal 16 (1962) pages 89 to 95, in which, however, the agar medium has been adapted to enable determinations of sulphas by the addition of trimethoprim (Merck Index 9th Ed. No. 9377). Sulphas are, generally speaking, compounds with a substituted or unsubstituted SO_2NH_2-group at the para site of a substituted or unsubstituted aniline nucleus, such as sulphamethoxazole [4-amino-N-(5-methyl-3-isoxazolyl) benzenesulphonamide]. In this process use is made of, inter alia, the thermophilic microorganism Bacillus stearothermophilus var. calidolactis, which is preferably incubated at about 60°C in order to avoid interferences by microorganisms present in the sample to be tested. In addition to the fact that fresh plates have to be prepared for each determination, the result of the test can be read not earlier than 6 hours after starting it.

The practice, however, needs a quicker test, using ready-for-use requirements, and giving a result within a few hours. In addition, the practice needs a test, which does not necessarily involve qualified laboratory personnel, and which may be carried out by, e.g. the truck driver transporting the milk from the farmer to the factory.

GB—A—1 467 439 describes a test starting indeed from ready-for-use requirements. That test gives a result with $1\frac{1}{2}$ to 4 hours, generally within 2 to 3 hours, and may be carried out by unqualified personnel, but the test is suitable for the determination of only antibiotics in biological liquids, such as milk, meat juice, serum and urine and shows too little sensitivity for the determination of sulphas.

According to the invention the test described in GB—A—1 467 439 is adapted for the determination of sulphas, and with the same test-duration of 2 to 3 hours.

Therefore the invention provides a process for the preparation of units for the determination of residues of antibiotics and sulphas in biological liquids, such as milk, meat juice, serum or urine, said units including an agar medium inoculated with spores of *Bacillus stearothermophilus var. calidolactis* and containing trimethoprim, characterized in that spores of *Bacillus Stearothermophilus var. calidolactis* (LMD 74.1) and trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)-1,3-pyrimidine] are introduced into an optionally buffered unmodified agar solution in concentrations of 10^5 to 10^8 spores per ml and 10 to 120 μg of trimethoprim per litre of agar medium, that optionally nutrients and an indicator suited for detecting growth of the microorganism, are added to the solution and the agar solution is then allowed to solidify in tubes, the process being carried out under such conditions that the spores stay alive but cannot germinate because of lack of nutrients and/or because of low temperature.

The units prepared according to the invention enable to obtain a result, within $1\frac{1}{2}$ to 4 hours, generally within 2 to 3 hours, whether a sample of biological liquid contains or does not contain an antibiotic or a sulpha in excess of a predetermined concentration. It has surprisingly been found that the test carried out with these units succeeds without the adaption of the medium, as reported by Gudding, so that the medium described in GB—A—1 467 439 may be used as such. It has also surprisingly been found that the time for reading the result is not necessarily extended. Thus, by choosing a suitable trimethoprim concentration and reading on acid formation or reduction, for instance according to the vertical diffusion test method of GB—A—1 467 439, the test time according to that method may be maintained. It is appreciated that Gudding used a trimethoprim concentration of 0.25 μg/ml, whereas, according to the invention, the trimethoprim concentration is lower, as indicated hereinbefore.

Trimethoprim appeared not or substantially not to influence the keepability of the spores. Furthermore, such a unit may be storable for more than a year.

Examples of units useful for the purpose of the invention are transparent tubes, single or in a set or combined to a block of translucent material provided with a number of holes shaped therein.

The nutrients necessary for the growth of the microorganism are preferably included in a tablet or in a disc of filter paper or anything like that. In a preferred embodiment of the invention the units as produced by the process described hereinbefore without the inclusion of nutrients are used in combination with nutrients in a tablet or filter paper disc adapted to be placed upon the agar medium before carrying out the determination. Nutrients, e.g. in a tablet, may also be included in the units beforehand, whereby preferably measures are taken to avoid moisture transport from the agar medium into the tablet. This may be done, e.g. by coating the tablet with a moisture-resistant layer, for example a wax, which coating must disappear during the test. A wax having a melting temperature of 35 to 55°C, preferably 40 to 45°C, is suitable for that purpose. The nutrients must contain at least an assimilable carbon

source, e.g. glucose, an assimilable nitrogen source, e.g. peptone, and a source of growth factor and minerals, e.g. yeast extract. If the nutrients are included in the agar medium the unit should be stored at temperatures below those where the spores germinate (2 to 10°C).

The indicator used is an acid-base indicator for a pH of about 5.5, preferably bromocresol-purple or phenolred, or a redox indicator, preferably 2,3,5-triphenyltetrazolium chloride or Brilliant black.

The strain *Bacillus stearothermophilus var. calidolactis* from which the spores are used in the process of the invention, has been deposited at the Laboratory of Microbiology of the Technical University of Delft under number LMD 74.1, where the strain is available to the public.

This microorganism is very sensitive for penicilins. It is growing fast and shows the additional advantages of the optimal growing temperature being so high that other microorganisms generally do not grow, resulting in only a small chance of those microorganisms being interfering. The microorganism further shows a high sensitivity for other antibiotics.

The preparation of the spore-containing agar medium is further described in GB—A—1 467 439.

In the determination of residues of antibiotics and sulphas in biological liquids, such as milk, meat juice, serum and urine, using the units as produced by the process of the invention, a predetermined amount of the sample to be tested is placed in the unit and is left there or removed after a sufficiently long time, e.g. 15 to 30 minutes for the diffusion of the residues of antibiotics and sulphas, subsequently if necessary the nutrients are placed on the agar medium, and the contents of the unit are incubated at or near the optimal temperature for the microorganism during a predetermined period after which the indicator-colour is observed, indicating the presence or absence of an antibiotic and/or sulpha above a certain minimum concentration. The test is very simple to be caried out, so that qualified personnel is not necessary for the test. The determination may be done in $1\frac{1}{2}$ to 4 hours, preferably 2 to 3 hours, after starting the test, which is markedly shorter than for the method described by Gudding.

Claims

1. Process for the preparation of units for the determination of residues of antibiotics and sulphas in biological liquids, said units including an agar medium inoculated with spores of *Bacillus Stearothermophilus var. calidolactis* and containing trimethoprim, characterized in that spores of *Bacillus Stearothermophilus var. calidolactis* (LMD 74.1) and trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)-1,3-pyrimidine] are introduced into an optionally buffered unmodified agar solution in concentrations of 10^5 to 10^8 spores per ml and 10 to 120 μg of trimethoprim per litre of agar medium, that optionally nutrients and an indicator suited for detecting growth of the microorganism, are added to the solution and the agar solution is then allowed to solidify in tubes, the process being carried out under such conditions that the spores stay alive but cannot germinate because of lack of nutrients and/or because of low temperature.

2. The units as produced by the process of claim 1 without the inclusion of nutrients, in combination with nutrients in a tablet or filter paper disc adapted to be placed upon the agar medium before carrying out the determination.

Revendications

1. Procédé de préparation de dispositifs pour la détermination de résidus d'antibiotiques et de sulfamidés dans des liquides biologiques, lesquels dispositifs comprennent un milieu à la gélose inoculé de spores de *Bacillus stearothermophilus var. calidolactis* et contenant du triméthoprime, caractérisé en ce que des spores de *Bacillus Stearothermophilus var. calidolactis* (LMD 74.1) et du triméthoprime [2,4-diamino-5-(3,4,5-triméthoxybenzyl)-1,3-pyrimidine] sont introduits dans une solution de gélose non modifiée, éventuellement tamponnée à des concentrations de 10^5 à 10^8 spores par ml et de 10 à 120 μg de triméthoprime par litre de milieu à la gélose, qu'éventuellement des agents nutritifs et un indicateur propre à la détection de la croissance du micro-organisme sont ajoutés à la solution et la solution de gélose est ensuite mise à solidifier dans des tubes, le procédé étant exécuté dans des conditions telles que les spores restent vivants, mais ne puissent germer en raison de l'absence d'agents nutritifs et/ou en raison d'une basse température.

2. Dispositifs préparés par le procédé suivant la revendication 1, sans apport d'agents nutritifs, en combinaison avec des agents nutritifs dans un comprimé ou un disque de papier-filtre propre à être posé sur le milieu à la gélose avant l'exécution de la détermination.

Patentansprüche

1. Verfahren zur Herstellung von Einheiten für die Bestimmung von Rückständen von Antibiotika und Sulfonamiden in biologischen Flüssigkeiten, wobei die Einheiten einen mit Sporen von Bacillus Stearothermophilus var. calidolactis geimpften und Trimethoprim enthaltenden Agarnährboden aufweisen, dadurch gekennzeichnet, dass man Sporen von Bacillus Stearothermophilus var. calidolactis (LMD 74.1) und Trimethoprim [2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-1,3-pyrimidin] in Konzentrationen von 10^5 bis 10^8 Sporen pro ml bzw. 10 bis 120 μg Trimethoprim pro Liter Agarnährboden in eine gegebenenfalls gepufferte unmodifizierte Agarlösung einbringt,

dass man gegebenenfalls Nährstoffe und einen für den Nachweis des Wachstums des Mikroorganismus geeigneten Indikator zu der Lösung zusetzt und die Agarlösung dann in Röhrchen erstarren lässt, wobei man das Verfahren unter solchen Bedingungen ausführt, dass die Sporen am Leben bleiben, aber wegen Mangels an Nährstoffen und/oder wegen der niedrigen Temperatur nicht keimen können.

2. Einheiten, hergestellt gemäss dem Verfahren nach Anspruch 1 ohne Einschluss von Nährstoffen, in Kombination mit Nährstoffen in einer Tablette oder Filtrierpapierscheibe, die dafür geeignet sind, vor der Ausführung der Bestimmung auf den Agarnährboden gelegt zu werden.